

Sulfate and Trace Metals Effects on Wild Rice Research - Phases II and III Quality Assurance
Project Plan

**Sulfate and Trace Metals Effects on Wild Rice Research - Phases II and III
Quality Assurance Project Plan**

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Date:

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A3 – Distribution List

Table 1 is a distribution list of people who will receive the approved QAPP, QAPP revisions, and any amendments. All documents will be delivered *via* emails.

Table 1. QAPP Distribution List

QAPP Recipient Name	Project Role Organization	Telephone number and Email address
John Pastor	Project Manager University of Minneosta, Duluth	218-726-7148 jpastor@d.umn.edu
Margaret Watkins	Grand Portage	(218) 475-2026 watkins@boreal.org
Nancy Schuldt	Fond du Lac	(218) 878-7110 NancySchuldt@fdlrez.com
Seth Moore	Grand Portage Quality Assurance Manager	(218) 475-2022 smoore@boreal.org
David Horak U.S. EPA, Region 5	USEPA Project Manager	(312) 353-4306 horak.david@epa.gov
John Dorkin	Water Quality Branch QA U.S. EPA, Region 5	(312) 886-1980 dorkin.john@epa.gov

A4 – Project/ Task Organization

Personnel who will be responsible/supporting the project:

John Pastor, is the Project Manager. He will be responsible for the overall project, including the design, development and implementation of the study, data analysis, and interpretation.

Margaret Watkins, Grand Portage Water Quality Specialist, and Nancy Schuldt, Fond du Lac Water Program Coordinator, will support the project and coordinate with US EPA Region 5. Seth Moore, the Grand Portage Environmental and Biology Department Director, will be the third-party quality assurance manager for the project.

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A5 – Problem Definition / Background

A 10 mg/l sulfate criterion has become controversial in northeastern Minnesota, because most of the existing and proposed ferrous and sulfide mines discharge water that is high in sulfates. Minnesota Statutes (174.05, CH 7050, Wild Rice Waters) currently mandates a 10 mg/l sulfate standard for industrial discharge between April and September, the period of seedling emergence and seed maturity and harvesting of wild rice. However, a recent environmental impact statement prepared for the Minnesota Pollution Control Agency has suggested that sulfate concentrations might be set as high as 250 mg/l without harm to wild rice, based on limited and mostly unpublished data rather than experimental evidence. Sulfate is the only criterion for the protection of wild rice in Grand Portage, Fond du Lac and Minnesota Water Quality Standards. Some trace metals may also be toxic to wild rice at relatively low concentrations. For example, copper is harmful to aquatic life at much lower concentrations than would be harmful to human health.

In 2009, Fond du Lac provided EPA Region 5, the state agencies, and tribal environmental staff in Minnesota the results of the first phase of this series of experiments. Dr. Pastor grew wild rice in 20 liter plastic buckets with sediment from Rice Portage Lake, a natural unpolluted wild rice bed on the Fond du Lac Reservation. Sulfate was added as Na_2SO_4 to achieve concentrations of 10, 100, and 300 mg/l, with an additional 0 mg/l control. They found that added sulfate, especially above the 100 mg/l level, significantly decreased wild rice root and total vegetative plant biomasses by 20% or more compared with biomasses of plants grown under low sulfate concentrations typical of natural waters. Although not statistically significant, seed production of plants grown under elevated sulfate concentrations were on average 17% lower than from plants grown under sulfate concentrations typical of natural waters. The significant decline in root biomass resulting from elevated sulfate concentrations may have resulted in nutrient stress to the plants as they may not have been able to exploit as large a volume of sediment to obtain nutrients.

In anaerobic environments, such as the organic rich sediment from the wild rice beds permanently submerged beneath water in wild rice beds and the buckets, sulfate is reduced to sulfide. Independent studies have shown that sulfide concentrations as low as 5-6 mg/l reduces oxygen export from roots to the surrounding sediment and thereby reduces water and iron uptake and lateral root emergence (Armstrong and Armstrong 2005). The reduction in total and root growth found by Armstrong and Armstrong as sulfate is reduced to sulfide parallels our finding of reductions in both total growth and root growth with added sulfate, suggesting that our results

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may also be due to sulfate reduction to sulfide.

A6 – Project / Task Description

We propose to continue the line of research conducted in 2009 at the University of Minnesota Duluth, investigating the protectiveness of the 10 mg/l sulfate criterion for wild rice waters, which is found in Fond du Lac, Grand Portage, and the state of Minnesota's water quality standards. This criterion has become controversial in northeastern Minnesota, because most of the existing and proposed ferrous and sulfide mines discharge water that is high in sulfates. Minnesota Statutes (174.05, CH 7050, Wild Rice Waters) currently mandates a 10 mg/l sulfate standard for industrial discharge between April and September, the period of seedling emergence, seed maturity, and harvesting of wild rice. However, a recent environmental impact statement for the Minnesota Pollution Control Agency has suggested that sulfate concentrations might be set as high as 250 mg/l without harm to wild rice, based on limited and mostly unpublished data rather than experimental evidence.

Additionally, in anaerobic environments, such as the organic rich sediment from the wild rice beds permanently submerged beneath water in wild rice beds and the buckets, sulfate is reduced to sulfide. Independent studies have shown that sulfide concentrations as low as 5-6 mg/l reduces oxygen export from roots to the surrounding sediment and thereby reduces water and iron uptake and lateral root emergence (Armstrong and Armstrong 2005). The reduction in total and root growth found by Armstrong and Armstrong as sulfate is reduced to sulfide parallels our finding of reductions in both total growth and root growth with added sulfate, suggesting that our results may also be due to sulfate reduction to sulfide.

We propose experiments for the summer of 2011 and 2012 to determine whether sulfate is reduced to sulfide levels in our buckets and whether the resulting sulfide levels are comparable to levels which reduce root growth found by Armstrong and Armstrong (2005). In order to bridge the gaps between no response to sulfate concentrations at 10 mg/l or less and decreasing plant growth at 100 to 300 mg/l, we propose to grow rice plants in water column sulfate concentrations of 0, 30, 60, 100, 150, and 300 mg $\text{SO}_4^{2-}/\text{l}$. The levels chosen bracket both the existing 10 mg/l statutory standard and the proposed 250 mg/l higher standard. Experiments will be performed at the University of Minnesota Duluth Research and Field Studies Station in Duluth, MN. Since climatic conditions (temperature and sunlight) during growing seasons present an uncontrolled variable between years, repeating the 10 mg/l and 100 mg/l level treatments will allow us to normalize growth responses in 2009 and 2010. This will enable us to determine a response curve across a sulfate gradient from 0 to 300 mg/l.

The effects of sulfate on wild rice growth may also depend in part on concentrations of

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other nutrients in the sediment and in the plant tissues themselves. Wild rice growth in natural unpolluted waters is limited by the supply of nitrogen (Walker et al 2010). If sulfate reduces root growth, then this may exacerbate nitrogen limitations because the root would only be able to occupy a smaller volume of sediment and therefore gain access to less available nitrogen. Symptoms of severe nitrogen limitation include reduced growth, reduced seed production, smaller seeds, and a general chlorosis of leaf tissues, all of which we have seen in our previous experiments with sulfate additions to wild rice, especially at sulfate concentrations of 300 mg/l. Therefore, plant samples will be analyzed for nitrogen concentrations and total nitrogen amounts per plant. These data will be analyzed for increased limitations using the approach of Timmer and Stone (1975).

Trace metals can also affect sulfate availability to plants. If sulfate is reduced to sulfides in the anaerobic sediments, iron or manganese could precipitate the sulfide, thus rendering it less available to plant roots. On the other hand, sulfate may also increase availability of iron, manganese, and other trace metals if the sulfate charge is balanced by that of free protons, this decreasing pH. In 2010, plants treated with sulfate concentrations of 0, 10, 100 and 300 milligrams per liter sulfate were analyzed for trace metals concentrations in the stems and seeds. The uptake of several metals was increased by sulfate treatments, but appeared generally limited to luxury uptake and did not result in toxicity or growth reduction. However, potassium uptake may have become slightly limiting to plant growth. In 2011, plants from the stock tanks will be analyzed for trace metals along with wild rice plants from waters that have been impacted by elevated sulfate and metals discharges collected in the 1854 ceded territories.

Field Setup and Experiment

Polyethylene stock tanks (Rubbermaid #4242, 378 L capacity, 132 cm long x 79 cm wide x 63 cm deep) will be fitted with overflow drain pipes, buried to ground level, and connected to 20 L polyethylene overflow buckets. Water tables will be set by the drain pipe at 23 cm above the sediment surface. The tanks will be partly filled with 10 cm of clean washed sand covered with 10 cm of surface sediment collected from natural wild rice beds from two lakes on the Fond du Lac Band of Lake Superior Ojibway Reservation in Carlton County, Minnesota. Ten to twenty cm of sediment over sand mimics the rooting depths we have observed in natural wild rice lakes. The sediments will be mixed in a large stock tank prior to distribution. Prior analysis of three volumetric samples of the mixed sediment indicate a homogenous material (% C = 12.18 \pm s.d. 1.00, % N = 1.07 \pm s.d. 0.02; Walker et al. 2010). Sediment bulk density was 0.27 g \cdot cm⁻³ \pm s.d. 0.01 (Walker et al. 2010). These values are comparable to those of other wild rice beds (Keenan and Lee 1988, Day and Lee 1989).

The tanks will be immediately filled with water after sediment additions to prevent the

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sediment from drying. Water will be added cautiously from a garden hose to prevent redistribution and suspension of sediment. During the growing season, water levels will be maintained by weekly additions of water to drain pipe heights or by allowing water to drain through the drain pipe set at 23 cm above the sediment surface into the overflow buckets. Water used to fill and supplement tank levels is obtained from a nearby wellpoint. Rainfall N concentrations as NO₃-N and NH₄-N ranged from 0.2 – 1.99 µg · mL⁻¹ while the NO₃-N and NH₄-N concentrations in the well water are always < 0.2 µg · mL⁻¹ (Walker et al. 2010). The sediments comprise an inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter will remain submerged year round with water levels set at approximately 20cm in late fall.

Wild rice will be planted in mid to late spring 2011 from seedlings germinated from cold stratified seeds obtained from the Grand Portage Reservation. End-of-season plant density in Minnesota wild rice lakes monitored by the 1854 Treaty Authority average 30-40 plants per square meter (Vogt 2010). We will plant seedlings at this density the first year, but as the experiment continues into subsequent years we will allow plant densities to fluctuate according to the seed production of each tank.

There will be five replicate tanks per treatment, for a total of 6 treatment levels X 5 replicates = 30 tanks. The water volume in the tanks from sediment surface to drain pipe height will be determined when filling the tanks. Sulfate will be added to water filled tanks as solutions of sodium sulfate in the appropriate concentrations as reported above (Fisher Chemical S421). Initially we will weigh a determined amount of Na₂SO₄, scoop 1 to 2 liters of water from the filled tank, dissolve the Na₂SO₄ in the container of water, then add it back to the tank with mixing. An example of this procedure would be as follows: given a tank volume of 150L, we would weigh 66.56g of Na₂SO₄, (molar wt. fraction of SO₄ is 0.676), dissolve in 2L of tank water (Na₂SO₄, solubility is 47.6g/L), then add back to the tank for a final concentration of 300 mg/L of SO₄. Subsequent adjustments to the sulfate concentration will be made every 2 weeks, after water sample analysis, with additions of 10g/L sulfate stock solution and well water to fill to drain pipe height. In all cases stock solution will be diluted with tank water before adding to the tanks to prevent abnormally high sulfate contact with the plants.

During the growing season we will sample the water from each tank every 2 weeks and the overflow buckets and rain gauge as needed and determine: sulfate concentrations with a Lachat Autoanalyzer by a barium chloride turbidimetric method (Lachat #10-116-10-1-A, USEPA/NPDES equivalent method), total nitrogen and phosphorous using persulfide digestion and Lachat Autoanalyzer methods (Lachat#10-115-01-1-A for orthophosphate and Lachat#10-107-04-1-C for nitrate). Sediment samples from the initial mixture and from each tank at the end of the growing season will be collected and analyzed for total carbon, nitrogen, and sulfur on a

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Thermo Electron Flash EA1112 elemental analyzer. Sediment samples from the initial mixture and from each bucket at the end of the growing season will be collected and analyzed for sulfide concentrations. Sulfate concentrations will be determined in sediments by the above procedure for water. Sulfide will be extracted with 1 M NaOH and analyzed by a methylthymol blue method (Lachat #12-116-29-3-A) on the autoanalyzer. In addition tank water pH and temperature will be measured in situ every 2 weeks using a portable meter.

Temperature, wind speed and direction, and precipitation will be monitored daily on site with an automated weather station equipped with a recording thermometer, radiosonde anemometer, and rain gauge. A temperature data logger (HOBO UA-002-08) will be placed on the sediment surface in one tank of each of the treatments, these will collect hourly temperatures throughout the growing season.

Five plants in each tank will be randomly chosen in early summer for detailed measurements throughout the growing season and sampling at the end of the growing season. Heights of these plants will be measured bi-weekly throughout the growing season. In late August to September, seeds will be collected by milking the seed head during the period of seed dispersal. At the end of the growing season these plants will be collected by gently excavating the root system from the sediment. Seeds will be collected by “milking” the seed head during the period of seed dispersal, and at the end of the season plants will be collected by suspending the sediment in the water column and freeing the root system. Seeds will be dried at 60° C, counted and weighed. Plants will be dried at 60° C, separated into root and stem/leaf sections, and then weighed. The plant samples will be milled and analyzed for total nitrogen on a Thermo Electron 1112 elemental analyzer. In addition the samples will be extracted to specification for ICP metal analysis by the Research Analytical Lab, Department of Soil, Water, and Climate at the University of Minnesota. In addition, all aboveground plant material will be collected from each tank and weighed along with a subsample taken to determine wet:dry ratios for moisture correction. All stems will be counted in these samples to determine end of growing season plant density. All aboveground plant material except for the five sample plants will be returned to each tank to mimic natural growing conditions to the extent possible.

Sediment samples from the initial mixture and from each tank at the end of the growing season will be collected and analyzed for sulfide concentrations. Sulfide will be extracted with 1 M NaOH and analyzed by a methylthymol blue method (Lachat #12-116-29-3-A) on the autoanalyzer. All data will be entered for statistical analysis in Excel spreadsheets. Mr. Brad Dewey, lab coordinator for J. Pastor, will oversee data entry and proof checking. All data files will be sent to appropriate managers and scientists at the Fond du Lac and Grand Portage Reservations. The effect of sulfate levels on wild rice seed, shoot, and root growth will be tested using a randomized complete block analysis of variance. Photographs will also be taken

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periodically during the growing season to visually document growth and condition of the stems and overall experimental design; digital images of these will also be provided to the reservations.

Fond du Lac covered the costs for the first year of this study, in year two Fond du Lac and Grand Portage equally shared the costs of the wild rice/sulfate research, and in year three, Grand Portage will cover all the costs for the experimental work and post-experimental analysis combined.

Table 2. Project Schedule Timeline

New tanks for this experiment will be installed at the University of Minnesota Duluth Field Research Station in late May 2011. Sediment will be obtained from Rice Portage Lake on the Fond du Lac Reservation at the same time. Wild rice seeds obtained from the Grand Portage Reservation will be planted in the tanks after they are installed with sediment. Plants will be thinned once they are at the floating leaf stage in approximately mid-late June. Height growth and water samples will be taken weekly during the growing season and water samples will be analyzed as they are obtained. Seeds begin ripening in late August, and seed collection will proceed from then until mid-late September, whereupon the rest of the plants will be harvested as above along with sediments. Plants will be weighed and analyzed during fall and winter of 2011-2012 and data analysis will proceed in spring 2012. A report will be prepared by May 2012.

A7 – Quality Objectives and Criteria

The Decisions

Five plants in each tank will be randomly chosen in early summer for detailed measurements throughout the growing season and sampling at the end of the growing season. Heights of these plants will be measured bi-weekly throughout the growing season. In late August to September, seeds will be collected by milking the seed head during the period of seed dispersal. At the end of the growing season these plants will be collected by gently excavating the root system from the sediment. In addition, all aboveground plant material will be collected from each tank and weighed along with a subsample taken to determine wet:dry ratios for moisture correction. All stems will be counted in these samples to determine end of growing season plant density. All aboveground plant material except for the five sample plants will be returned to each tank.

Inputs to the Decisions

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Decisions on modifications to the experimental design will be made by John Pastor in consultation with Margaret Watkins (Grand Portage Reservation) and Nancy Schuldt (Fond du Lac Reservation). It is not anticipated at this time that there will be any major changes to the experiment but unforeseen circumstances, such as outbreaks of rice worm, may arise from time to time that require additional measurements and modifications to the sampling regime.

Study Boundaries

The sediment for the experiments will be obtained from Rice Portage Lake, a large natural wild rice lake on the Fond du Lac Reservation in Minnesota. These sediments have been used on other experiments on wild rice growth and ecology (Walker et al. 2010). The seeds will be obtained from natural wild rice stands on the Grand Portage Reservation. Water for the tanks will be supplied from an artesian well at the University of Minnesota Field Experiment Station; concentrations of nitrate and sulfate in this water are consistently below detection limit.

A8 – Special Training / Certification

Study participants will be involved in wild rice sampling based on EPA approved methodology and currently approved QAPP.

The lab personnel at University of MN, Duluth will be trained for proper techniques and bio-safety.

A9 – Documents / Records

- The water samples collected from the tanks will be documented using established tracking methods of the Pastor lab.
- All records will be kept in the laboratory. The Grand Portage Water Quality Specialist, the Grand Portage Biology and Environmental Department Director, and Fond du Lac Water Program Coordinator will also receive and retain copies.
- All data will be entered for statistical analysis in Excel spreadsheets
- Mr. Brad Dewey, lab coordinator for J. Pastor, will oversee data entry and proof checking.
- All data files will be sent to appropriate managers and scientists at the Fond du Lac and Grand Portage Reservations
- The effect of sulfate levels on wild rice seed, shoot, and root growth will be tested using a randomized complete block analysis of variance.

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- Photographs will also be taken periodically during the growing season to visually document growth and condition of the stems and overall experimental design; digital images of these will also be provided to the reservations.
- The approved QAPP will be recorded and submitted to people on the distribution list (Table 1).

B1 – Sampling Process Design

All sampling will follow the established general rules:

Temperature, wind speed and direction, and precipitation will be monitored daily on site with an automated weather station equipped with a recording thermometer, radiosonde anemometer, and rain gauge. A temperature data logger (HOBO UA-002-08) will be placed on the sediment surface in one tank of each of the treatments, these will collect hourly temperatures throughout the growing season.

There will be five replicate tanks per treatment, for a total of 6 treatment levels X 5 replicates = 30 tanks. The water volume in the tanks from sediment surface to drain pipe height will be determined when filling the tanks. Sulfate will be added to water filled tanks as solutions of sodium sulfate in the appropriate concentrations as reported above (Fisher Chemical S421). Initially we will weigh a determined amount of Na₂SO₄, scoop 1 to 2 liters of water from the filled tank, dissolve the Na₂SO₄ in the container of water, and then add it back to the tank with mixing. An example of this procedure would be as follows: given a tank volume of 150L, we would weigh 66.56g of Na₂SO₄, (molar wt. fraction of SO₄ is 0.676), dissolve in 2L of tank water (Na₂SO₄, solubility is 47.6g/L), then add back to the tank for a final concentration of 300 mg/liter of SO₄. Subsequent adjustments to the sulfate concentration will be made every 2 weeks, after water sample analysis, with additions of 10g/L sulfate stock solution and well water to fill to drain pipe height. In all cases stock solution will be diluted with tank water before adding to the tanks to prevent abnormally high sulfate contact with the plants.

B2 – Sampling Methods

Sample Collection

Five plants in each tank will be randomly chosen in early summer for detailed measurements throughout the growing season and sampling at the end of the growing season. Heights of these plants will be measured bi-weekly throughout the growing season. In late August to September, seeds will be collected by milking the seed head during the period of seed dispersal. At the end of the growing season these plants will be collected by gently excavating

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the root system from the sediment. In addition, all aboveground plant material will be collected from each tank and weighed along with a subsample taken to determine wet:dry ratios for moisture correction. All stems will be counted in these samples to determine end of growing season plant density.

Isolation of chemicals from water

During the growing season we will sample the water from each tank every 2 weeks and the overflow buckets and rain gauge as needed and determine: sulfate concentrations with a Lachat Autoanalyzer by a barium chloride turbidimetric method (Lachat #10-116-10-1-A, USEPA/NPDES equivalent method), total nitrogen and phosphorous using persulfide digestion and Lachat Autoanalyzer methods (Lachat#10-115-01-1-A for orthophosphate and Lachat#10-107-04-1-C for nitrate). Sediment samples from the initial mixture and from each tank at the end of the growing season will be collected and analyzed for total carbon, nitrogen, and sulfur on a Thermo Electron Flash EA1112 elemental analyzer. Sediment samples from the initial mixture and from each bucket at the end of the growing season will be collected and analyzed for sulfide concentrations. Sulfate concentrations will be determined in sediments by the above procedure for water. Sulfide will be extracted with 1 M NaOH and analyzed by a methylthymol blue method (Lachat #12-116-29-3-A) on the autoanalyzer. In addition tank water pH and temperature will be measured in situ every 2 weeks using a portable meter.

Storage

Seeds will be collected by milking the seed head during the period of seed dispersal, and at the end of the season plants will be collected by suspending the sediment in the water column and freeing the root system. Seeds will be dried at 60° C, counted and weighed. Plants will be dried at 60° C, separated into root and stem/leaf sections, and then weighed. The plant samples will be milled and analyzed for total nitrogen on a Thermo Electron 1112 elemental analyzer. In addition the samples will be extracted to specification for ICP metal analysis by the Analytical Lab, Department of Soil, Water, and Climate at the University of Minnesota.

B3 – Sample Handling and Custody

All samples collected will be labeled to identify the sample for database record. The labels will include location, data, time, and other information documents as required. Sample labels must be properly completed to include the sample's field identification number. Labels shall be printed and affixed to the outside wall of the sample container in the lab, prior to going to the field.

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All laboratory-identified samples will be labeled for database record. The labels will include the abbreviation of date, method, location, size, and media.

B4 – Analytical Methods

Sediment samples from the initial mixture and from each bucket at the end of the growing season will be collected and analyzed for sulfide concentrations. Sulfide will be extracted with 1 M NaOH and analyzed by the methylthymol blue method (Lachat #12-116-29-3-A) on the autoanalyzer. Plant heights will be measured bi-weekly throughout the growing season, seeds will be collected by milking the seed head during the period of seed dispersal, and at the end of the season plants will be collected by suspending the sediment in the water column and freeing the root system.

Seeds will be dried at 60° C, counted and weighed. Plants will be dried at 60° C, separated into root and stem/leaf sections, and then weighed. The plant samples will be milled and analyzed for total nitrogen on a Thermo Electron 1112 elemental analyzer.

In addition the samples will be extracted to specification for ICP metal analysis by the Research Analytical Lab, Department of Soil, Water, and Climate at the University of Minnesota.

B5 – Quality Control

The methods of analysis are all industry standard methods. The reference numbers are:

- Lachat Method 10-116-10-2-A MTB Sulfate in Water
- Lachat Method 12-116-29-3-A MTB Sulfide in Soil Extracts
- Sulfate Effects on Wild Rice Research - Phases II and III QAPP
- Plant Nitrogen is run on CE Elantech Flash EA112 Elemental Analyzer
- Plant Metals run by an Inductively Coupled Argon Plasma Optical Emission Spectrometer are operated by the Research Analytical Laboratory, Department of Soil, Water, and Climate, University of Minnesota using methods of: Fassel, V.A., and R.N. Kniseley. Nov. 1974. Inductively Coupled Plasma Optical Emission Spectroscopy. Anal. Chem. 46 (13):1110A-1120A; see also: Dahlquist, R.L. and J.W. Knoll. 1978. Inductively Coupled Plasma-Atomic Emission Spectrometry: Analysis of biological materials and soils for major trace, and ultra-trace elements. Appl. Spectroscopy 32:1-30. ICP: ARL (Fisons) Model 3560 ICP-AES Ref.

B6 – Instrument / Equipment Testing, Inspection, Maintenance

Standard instruments and supplies for plant growth and sulfide isolation will be maintained in good condition in John Pastor's laboratory.

Any images will be inspected in John Pastor's laboratory by visual inspection of 10 % of the total images.

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The database system will be maintained by John Pastor's laboratory.

B7 – Instrument / Equipment Calibration and Frequency

The frequency and procedures used to calibrate equipment including incubators, shakers, waterbaths, spectrophotometers, pipettes and lasers are dictated by the University of Minnesota laboratory Standard Operating Procedure

B8 – Inspection / Acceptance Requirements for Supplies and Consumables

Standard procedure for inspection/acceptance requirements for supplies will be routinely performed by trained technical personnel in John Pastor's laboratory.

B9 – Non-Direct Measurements

B10 – Data Management

- The field samples will be recorded as they are collected.
- The water samples collected from the tanks will be documented using established tracking methods of the Pastor lab.
- All records will be kept in the laboratory. The Grand Portage Water Quality Specialist and Fond du Lac Water Program Coordinator will also receive and retain copies.
- All data will be entered for statistical analysis in Excel spreadsheets
- Mr. Brad Dewey, lab coordinator for J. Pastor, will oversee data entry and proof checking.
- All data files will be sent to appropriate managers and scientists at the Fond du Lac and Grand Portage Reservations
- The effect of sulfate levels on wild rice seed, shoot, and root growth will be tested using a randomized complete block analysis of variance with prescribed sulfate level as the main effect. The effects of sulfate additions on sediment sulfide concentrations and plant tissue nitrogen and trace metal concentrations will also be tested using a randomized complete block analysis of variance with prescribed sulfate level as the main effect.
- Photographs will also be taken periodically during the growing season to visually document growth and condition of the stems and overall experimental design; digital images of these will also be provided to the reservations.
- The approved QAPP will be recorded and submitted to people on the distribution list (Table 1).

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C1 – Assessment and Response Actions

Water sampling quality assessment

Classification accuracy

C2 – Reports to Management

The Principal Investigator will submit annual reports to the Grand Portage and Fond du Lac Environmental Managers, who in turn will inform the EPA Project Officer of the results. The final report will be submitted to the Grand Portage and Fond du Lac Environmental Managers, with a copy to the EPA technical contact no later than 45 days before the end of the project period for review and comment.

Deliverable: Final research report on the experimental sulfate treatments and post-experimental plant tissue analysis.

D1 – Data Review, Verification and Validation

- All data will be entered for statistical analysis in Excel spreadsheets
- Mr. Brad Dewey, lab coordinator for J. Pastor, will oversee data entry and proof checking.
- The effect of sulfate levels on wild rice seed, shoot, and root growth will be tested using a randomized complete block analysis of variance.
- Photographs will also be taken periodically during the growing season to visually document growth and condition of the stems and overall experimental design; digital images of these will also be provided to the reservations.

D2 – Verification and Validation Procedures

Wild rice sampling will be performed by lab members under this QAPP. The sample preparation will be supervised, verified, and validated by John Pastor.

Any images will be verified and validated by John Pastor, for patterns, changes, etc. Visual verification and validation will be performed for each batch of images by visually inspecting 10% of images.

All materials created from this project will be peer reviewed before publication.

Furthermore, results from this effort may be presented at an annual conference and submitted for journal publication.

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Plant heights will be measured bi-weekly throughout the growing season, seeds will be collected by milking the seed head during the period of seed dispersal, and at the end of the season plants will be collected by suspending the sediment in the water column and freeing the root system. Seeds will be dried at 60° C, counted and weighed. Plants will be dried at 60° C, separated into root and stem/leaf sections, and then weighed. The plant samples will be milled and analyzed for total nitrogen on a Thermo Electron 1112 elemental analyzer. In addition the samples will be extracted to specification for ICP metal analysis by the Research Analytical Lab, Department of Soil, Water, and Climate at the University of Minnesota. Fond du Lac covered the costs for the first year of this study, in year two Fond du Lac and Grand Portage equally shared the costs of the wild rice/sulfate research, and in year three, Grand Portage will cover all the costs for the experimental work and post-experimental analysis combined.

Standards for the sulfate and sulfite analyses will be prepared the day that samples are run. A complete set of standards bracketing the expected range of concentrations is run for each tray of 40 samples, with a check standard run every 10 samples. For the tissue nitrogen analyses, we use LECO Corporation #502-278 Rice Flour certified C, H, N, S Standard for daily calibration of the instrument and as a periodic check standard.

Trace metals can also affect sulfate availability to plants. If sulfate is reduced to sulfides in the anaerobic sediments, iron or manganese could precipitate the sulfide, thus rendering it less available to plant roots. On the other hand, sulfate may also increase availability of iron, manganese, and other trace metals if the sulfate charge is balanced by that of free protons, this decreasing pH. In 2010, plants treated with sulfate concentrations of 0, 10, 100 and 300 milligrams per liter sulfate were analyzed for trace metals concentrations in the stems and seeds. The uptake of several metals was increased by sulfate treatments, but appeared generally limited to luxury uptake and did not result in toxicity or growth reduction. However, potassium uptake may have become slightly limiting to plant growth. In 2011, plants from the stock tanks will be analyzed for trace metals along with wild rice plants from waters that have been impacted by elevated sulfate and metals discharges collected in the 1854 ceded territories.

Exploratory Trace Metals Analysis for Wild Rice plants collected in the 1854 ceded territory

Wild rice plants will be collected by the 1854 Treaty Authority from wild rice sampling locations that were identified by maps and field observations. Sampling will be done only in locations with sufficient wild rice plants present to avoid impact to health of stand. The site name, GPS coordinates, date, time, crew member names, and site description/comments will be recorded in the field on datasheet. Wild rice plants will be grabbed by the stalks and pulled slowly to remove plant and roots from sediment. Collection will include 5 plants at each location. Any sediment remaining on the roots will be immediately rinsed off in the lake/river.

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Plants will be placed in sample bags (2.5 gallon zip-loc bags) and labeled with permanent marker with date, time, and location. Sample bags will be stored in cooler with ice packs after collection in the field for transportation back to the office. Sample bags will be stored in refrigerator at office and delivered to the UMD laboratory the next day.

Trace metals analysis will be conducted by the U of M and will include plants collected by the 1854 Treaty Authority and wild rice plants grown in the stock tanks at UMD. Trace metals analyzed will include Aluminum, Boron, Calcium, Cobalt, Chromium, Copper, Iron, Potassium, Magnesium, Manganese, Molybdenum, Sodium, Nickel, Potassium, Zinc. The concentrations of trace metals found from each site will be compared to trace metals concentrations found in the plants from other sites and plants grown in the stock tanks. We will also be comparing trace metals concentrations with water column sulfate concentrations.

Deliverable: Final research report on the experimental sulfate treatments and post-experimental plant tissue analysis.

Outputs: Data and analysis that can be shared with other tribes, state and federal agencies, filling a critical science gap in our regulatory programs.

Outcomes: Clearer understanding of the effects of sulfate on wild rice growth and reproduction, and the protectiveness of the existing tribal and state water quality criterion. Grand Portage will use the data and analysis to revise/refine our federally approved Water Quality Standards criteria for the protection of wild rice.